

MORPHOLOGY, PHYSIOLOGY, GENETICS, ENIGMAS, AND STATUS OF
AN EXTREMELY RARE TREE: MUTANT TANOAK

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ABSTRACT

Important physical characteristics, morphological attributes, physiological functions, and genetic properties of mutant tanoak, *Notholithocarpus densiflorus* f. *attenuato-dentatus* (Fagaceae), and normal tanoak, *Notholithocarpus densiflorus* (Hook. & Arn.) Manos, Cannon & S. H. Oh, were studied on the Challenge Experimental Forest in Yuba Co., California in an attempt to explain the cause of the mutation and to determine where in the tree it was manifest. Leaves, stomata, trichomes, foliar nutrients, photosynthesis, transpiration, internal moisture stress, DNA, and genetics (metabolomics) all were examined in detail. In some instances, the plant part or the process favored the mutant; in others, the normal tanoak exceeded. Susceptibility to *Phytophthora ramorum*, the sudden oak death pathogen (SOD) was similar. No all-encompassing functional difference for either type was indicated, other than the size and shape of the leaves and the metabolites in them. We know the two tanoak types differ genetically, but more complete genomic analysis is needed to pinpoint the cause of the mutation. Some thought-provoking enigmas concerning the morphology and physiology of tanoak are presented along with the status (number of plants and location) of the rare mutant.

Key Words: DNA, ecology, genetics, mutant and normal tanoak, *Lithocarpus densiflorus*, *Notholithocarpus densiflorus*, physiology, status.

The mutant form of tanoak, *Notholithocarpus densiflorus* f. *attenuato-dentatus* (Fagaceae) (Tucker et al. 1969) (Fig. 1), is extremely rare in a natural setting. Knowledge of this mutant, the cause of its condition, and its future as a tanoak derivative not only could add to our understanding of the genus *Notholithocarpus*, but also might have practical value by giving a clue for lessening the impact of sudden oak death (SOD).

Tanoak is an evergreen hardwood that is considered a link between the chestnut (*Castanea*) and the oak (*Quercus*) (Sudworth 1967). It has flowers like the chestnut and acorns like the oak. Over the years, taxonomists have had difficulty classifying the species. According to the synonymy in Little (1979), tanoak was listed as *Quercus densiflora* in 1840, *Pasania densiflora* in 1867, and *Lithocarpus densiflorus* in 1916. Recently, another genus classification has been established (*Notholithocarpus*; Manos et al. 2008) that separates the single North American tanoak from the far-eastern genus *Lithocarpus*.

Tanoak is a medium-sized tree that grows best on the moist, west-facing slopes of the Cascade Range and Sierra Nevada in Oregon and

California southward to Santa Barbara Co. It usually occurs in a complex mixture with conifers and other hardwoods or in pure, even-aged stands (Tappeiner et al. 1990). Tanoak forms single trees or clumps of two to five trees that originate from root-crown sprouts.

Tanoak is a member of a large group of plants called “broad sclerophylls,” which are considered to be well adapted to a wide variety of environments (Mooney and Dunn 1970; McDonald 1982). At least part of the evidence for this classification is found in the fossil record—paleobotanists have traced this species back 12–26 million years to a period during the late Oligocene to mid-Miocene epochs (Cooper 1922). Tanoak has survived volcanism, glaciation, upheaval, and subsidence in at least part of its present range. Consequently, adaptation to heat, cold, and drought are likely to be part of tanoak’s genetic makeup. Having existed for millions of years, tanoak is considered to be an evolutionary species or lineage—“It is a lineage, an ancestral-descendant sequence of populations existing in space and in time.” (Grant 1971, p. 38).

Tree seedlings with peculiar leaves and a low, shrubby form (Fig. 1) were first discovered on the



FIG. 1. At about 2 m in height, this was the largest mutant tanoak on the Challenge Experimental Forest, Yuba Co., California in August 1967. It disappeared shortly after this photo was taken. (Photo credit: Tucker et al. 1969).

USDA Forest Service's Challenge Experimental Forest (maintained by the Pacific Southwest Research Station, Albany, CA) in Yuba Co., California in January 1962. Believed to be some form of mutant, the identity of these trees was not immediately apparent.

Their location, however, gave a clue to their origin. About 20 mutant seedlings were found scattered beneath a large, open-growing tanoak "mother tree" along with scores of normal seedlings. To prove tanoak parentage, acorns were gathered beneath this and nearby trees in the fall of 1965 and germinated in pots in a greenhouse. Of the 45 acorns that germinated, one clearly was a mutant (Tucker et al. 1969).

Why were the mutants so weak and slow growing? What was causing their formation? What were the odds that all of them would die and be lost to botanists and other interested disciplines, possibly forever?

A chromosomal aberration was suspected, and squash preparations to examine chromosome counts were carried out on several occasions. All attempts were unsuccessful (Tucker et al. 1969). A

likely hypothesis was advanced by Robert Echols, a Pacific Southwest Research Station geneticist, who suggested that the aberration, a sublethal recessive condition, could have been caused by selfing (self-pollination).

By 1969, 10 tanoak mother trees with mutant seedlings nearby were located on or near the Experimental Forest. These trees were quite similar with fully developed, wide-spreading crowns and a shaded, deep organic layer beneath them. Almost all mutant seedlings were 3–40-cm tall and appeared to be in poor health with little or no growth.

In 1974, a mother tree with both mutant and normal seedlings beneath was visited again, and not a single mutant could be found. The spring of 1973 was particularly cold, and the abnormal temperatures could have been lethal to the mutants, but not to the normal seedlings. Over the years, all of the known mother trees at Challenge died, were inadvertently lost to wood cutters, or could not be relocated.

As interest increased, the search for mutant tanoaks accelerated, and seedlings beneath hundreds of tanoaks were examined both on the Experimental Forest and throughout most of the species' natural range. Botanists and silviculturists on many national forests were questioned, and a herbarium specimen was shown to several, but no additional mutants were found.

The objective of this paper is to report our findings on the physical characteristics, morphological attributes, physiological functions, and genetic properties of the mutant tanoak in an attempt to characterize the mutation, learn where it manifests, and understand its population dynamics. Another objective was to look for something (anything) in the mutant that might help curtail the ravages of SOD. To help achieve these objectives, we compared the mutant to a normal tanoak of about the same age and development growing nearby. Any differences that we found could then help to isolate critical elements in the mutant, and possibly lead to a genetic explanation of its mutancy.

METHODS

The study was located on the Challenge Experimental Forest in north-central California (Yuba Co.), T19N R7E, MDM sect. 20. The forest cover type in the vicinity of the study site is Pacific ponderosa pine–Douglas-fir (SAF type 244) (McDonald 1980). Several conifer and hardwood species characterize this type, and species near the sample trees included ponderosa pine (*Pinus ponderosa* P. Lawson & C. Lawson. var. *ponderosa*), Pacific madrone (*Arbutus menziesii* Pursh), and many tanoak seedlings, saplings, and trees. (Scientific and common names of



FIG. 2. Mutant tanoak tree sampled in this study on the Challenge Experimental Forest, Yuba Co., California.

trees are from Little [1979].) In terms of ecological subregions of California, the area corresponds to section M261E Sierra Nevada and the granitic and metamorphic hills subsection (Miles and Goudey 1997).

The original forest was logged (at least for the largest and best conifer trees) from about 1860–1890 (McDonald and Lahore 1984). Thus, logging and the inevitable fires caused the current forest to be a mosaic of even-age, second-growth stands. Summers on the Experimental Forest are hot and dry; the winters cool and moist. The mean annual temperature is 16°C. The growing season is about 200 days. Average annual precipitation is 1720 mm with 94% falling between October and May (USDA Forest Service unpublished). A typical soil series (Aiken) grades from loam to clay-loam with depth, and is deep, moderately well drained, and quite fertile.

By 2009, no mutant seedlings at Challenge could be located anywhere, and only three larger entities remained. These consisted of a slender moribund tree with a 50° lean, a clump of root-crown sprouts, and a single upright tree.

The best chance for achieving the objectives was to sample the most representative of the mutants. The severely leaning moribund specimen was not suitable, and the root-crown sprouts had severe limitations. Of the seven

root-crown sprouts, five were alive, although one was top-dead with only two living lower branches. The remaining four trees had breast-height diameters that ranged from 2.2–7.5 cm and heights from 5–11 m. The age of the tallest root-crown sprout was 22 yrs at breast height and 26 yrs at 30 cm above mean ground line. All were leaning 20–40° away from a large pine 1.5 m to the north. Another negative was that no similar-sized, normal tanoaks were nearby.

Plainly, the single upright mutant tree was from seed and not a root-crown sprout. It and several similar-sized, normal tanoaks were located at the 825 m elevation on level ground. One normal tanoak was randomly chosen. Both the mutant and normal sample trees lean about 20° into a small opening, and although shorter than surrounding trees, are not overtopped.

Sampling of the mutant tree began on August 14, 2008, when basic data on it and the normal tanoak tree nearby were taken (Fig. 2). Both trees were bored with a standard increment borer at two places on the stem, and the rings counted to determine age.

Leaves from the mutant and the normal tanoak were gathered from small branches cut at mid-crown with a long-handled pruning pole. Sampling time was from 1000 to 1400 hrs PST. A sample of leaves was gathered from more than 30

normal tanoak trees in the surrounding area for DNA analysis. Additional leaves were gathered for other tests as needed.

To determine stomatal density and aperture length, three leaves from both the normal and mutant tanoaks were randomly selected. Stomatal density was determined for both the upper and lower leaf surfaces using imprints made with transparent nail varnish. The number of stomata per mm^2 was then recorded in three randomly selected areas and measured under a stereomicroscope equipped with an ocular micrometer. Stomatal aperture lengths, defined as the distance between the junctions or ends of the guard cells, were also measured on ten stomata from each leaf type. Only stomata from the lower or underside of the leaves were used for this measurement.

We were also interested in the trichomes on the underside of the leaves. Trichomes are a beneficial adaptation for the species because their fuzzy nature is both anathema to hungry herbivores (King and Radosevich 1980), as well as an effective means for reducing transpiration. Sampling was accomplished by pressing a small piece of electrical tape to the midrib and the leaf blade, and then sticking the tape to a standard microscope slide. Sampling intensity was 10 leaves from each tanoak type. Trichomes were counted along two transects on the midrib and two plots on the blade with a dissecting scope. Each transect was a long rectangle with an area of 2.8 mm^2 , and the blade plots were 16.3 mm^2 in size.

Foliar analysis followed standard protocols (Bremner 1970) with drying, grinding, and digesting with acid (Kjeldahl) to determine the percentages of each macronutrient (nitrogen, phosphorus, potassium), and a mass spectrometer to indicate parts per million of the micronutrients and metals (aluminum, boron, calcium, copper, iron, magnesium, sodium, sulfur, sodium, and zinc) in the tanoak leaves.

Photosynthetic gas exchange was measured on August 14 for both the mutant and normal tanoak leaves with a Li-Cor 6400 portable photosynthesis system with a red/blue LED light source and CO_2 injector (Li-Cor Inc. Lincoln, NE), by setting photosynthetically active radiation (PAR) from 1500 to 1000, 500, 200, 100, 50, and $0 \mu\text{mol m}^{-2} \text{ s}^{-1}$. We let the leaves acclimate in the cuvette for three minutes before each measurement. The temperature within the cuvette was maintained near the ambient air temperature (31°C). A constant CO_2 concentration at $380 \mu\text{mol mol}^{-1}$ was supplied with fixed air flow of 500 mol s^{-1} . Data were taken from two healthy leaves on each tree. We fit these data with the non-rectangular hyperbolic model developed by Hanson et al. (1987).

During the measurement of gas exchange, internal water potential was measured with a

pressure chamber (PMS Instrument Company, Corvallis, OR) on small twigs with leaves attached at each sampling time. In addition, we measured average soil moisture content from the soil surface to the 20 cm depth with a CS 620 Water Content Hydrosense (Campbell Scientific, Inc., Logan, UT) at four locations around both the mutant and normal trees. It was 7.9% with a standard deviation of 1.7% by volume.

The first approach we took to understand the genetic differences between the mutant and normal tanoaks was a relatively new technique called metabolomics (Macel et al. 2010). It involves identifying the metabolites found in the leaf tissue of both the mutant and normal tanoaks. If the mutation impacts a metabolic pathway, differences between the two forms of tanoak can be determined, and these could suggest what gene(s) are involved in the mutation. Sampling intensity was six leaves from the mutant and two leaves each from three normal trees. Additional normal trees were included in this part of the study, as we wanted to sample the range of metabolites that were present in surrounding trees. Leaves were stored on dry ice until transported to the UC Davis Metabolomics Core Facility where they were prepared and analyzed using standard protocols. Based on mass spectrometry, peaks for a range of metabolic products were produced and analyzed using principal components analysis (PCA). This technique is ideal for summarizing multivariate data.

The second genetic approach we took was to examine variation at putatively neutral microsatellite loci. DNA was extracted from the leaves of the mutant tanoak and from those of many normal trees nearby and analyzed in the laboratory of Richard Dodd at UC Berkeley. Extraction protocols as well as chloroplast and nuclear DNA marker analyses followed Nettle et al. (2009). Five polymorphic chloroplast markers and 11 nuclear microsatellite markers were scored.

In the mid-1990s a new disease affecting tanoak surfaced on the West Coast of California and spread rapidly to several counties in coastal California and Oregon. It is called sudden oak death (SOD) and is caused by a fungus-like microorganism named *Phytophthora ramorum*. In some infected trees, cankers appear, the bark splits, the wound oozes, and as early as 6–24 mos after infection, the tree dies. Thus far, the disease has not spread to wildlands in drier locales east of the Cascade Range and the Sierra Nevada.

To determine if the mutant tanoak was more or less susceptible than normal tanoaks to SOD, leaves from the mutant and nearby normal tanoak trees were inoculated with the pathogen and tested for susceptibility using a detached leaf assay (Hayden et al. 2011). This work was performed in the laboratory of Matteo Garbelotto at the University of California, Berkeley.

RESULTS AND DISCUSSION

Physical Characteristics

Branch angle, bark roughness, and bark color were similar for the two sampled trees. Diameter at breast height (1.4 m), diameter at 30 cm above mean ground line, and tree height were quite similar as well (Table 1). Both sample trees grew slowly when young and then faster after reaching breast height. Each eventually produced flowers, but no acorns.

The unique physical characteristics and morphological attributes of the two types of tanoak leaves and their stomata and trichomes have an important physiological role. Leaves from the normal tanoak were shorter and wider and had three times as many stomata as those from the mutant, but the number of trichomes was significantly fewer. In general, more open stomata mean more carbon dioxide intake, and a higher potential for increased transpiration, but the higher trichome density on mutant leaves could elevate the boundary layer and lower the ability of the wind to "pull" moisture from them.

Morphological Attributes

Leaves of the two forms of tanoak constituted the place where the most obvious differences became manifest (Fig. 3). Leaves from the mutant tree were 20% longer and 57% narrower than leaves from the normal tanoak tree (Table 2). Both dimensions differed statistically ($P < 0.05$), with the differences directly translated into significantly smaller leaf areas and lower, but not significantly lower, dry weights of the leaves from the mutant tree. The width of the mutant leaves was narrow regardless of length, but the width of the leaves from the normal tanoak tree increased in proportion to length.

The distinct difference in leaf shape suggests a possible biophysical advantage for the mutant type in leaf level temperature and thus transpirational water loss. We used a simplified energy balanced equation where incident radiation, emissivity, absorption, and transpiration rate ($4 \text{ mmol m}^{-2} \text{ s}^{-1}$) were considered equivalent for leaves from both trees (Nobel 2005). At ambient temperatures above 30°C and low wind speeds (1 m s^{-1}), leaf temperature of the mutant

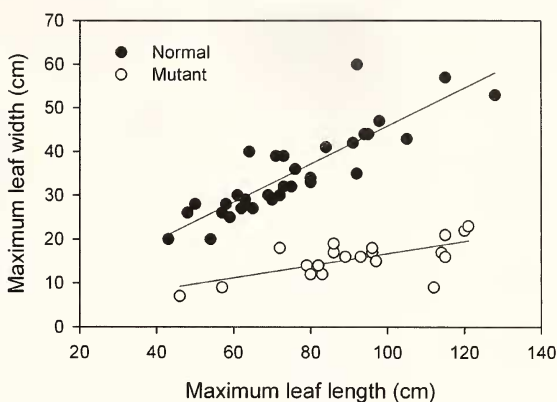


FIG. 3. Relationship between width and length of mutant and normal tanoak leaves collected from individual trees near the Challenge Experimental Forest, Yuba Co., California.

tanoak was nearly 2°C less than leaves of the normal tree.

For leaves from both trees, only a few stomata were observed on the upper side of the leaves and many more on the lower side. For the upper side, a standard t-test indicated no statistically significant difference in stomatal density between the two tanoak types, but for the lower side, three times as many stomata on the average normal leaf were present versus that of the mutant—a highly significant difference (Table 3). No statistical difference between the two tanoak types was found for aperture length with the overall average being $25.0 \pm 2.5 \mu\text{m}$.

The "center and arms" configuration of the tanoak trichomes, their curving, twisting shape, and their high density indicate an underside leaf surface that could influence internal water relations of the two tree types (Fig. 4). A standard two-tailed statistical test showed significant differences between the mutant and the normal trees. Leaves from the mutant tree had significantly more trichomes on both the midrib ($P < 0.0001$) and the blade ($P < 0.0002$) than did the normal leaves (Fig. 5).

Foliar analysis indicated no statistically significant differences between the two tanoak trees in the percentage of nitrogen, phosphorus, or potassium (Table 4), but did show significant differences ($P < 0.05$) in several micronutrient elements (boron, calcium, copper, manganese, and zinc). Nearly all elements in the mutant were substantially less than in the normal tree. Although there were no differences in the elemental concentration of nitrogen, the concentration per unit area was 60% greater in leaves from the mutant tree than in the normal leaves (0.08 versus $0.05 \text{ mg kg}^{-1} \text{ cm}^{-2}$).

The lower internal leaf temperature of the mutant would decrease the leaf-air water vapor concentration gradient, lower transpirational

TABLE 1. PHYSICAL CHARACTERISTICS OF MUTANT AND NORMAL TANOAK TREES. Challenge Experimental Forest, Yuba Co., California.

Tree	Age at breast height (years)	Age at 30 cm (years)	Height (m)	Diameter (cm)
Mutant	22	26	10.9	9.0
Normal	20	28	11.8	11.4

TABLE 2. MEAN MORPHOLOGICAL CHARACTERISTICS (AND STANDARD DEVIATION) OF LEAVES FROM NORMAL AND MUTANT TANOAK TREES. Challenge Experimental Forest, Yuba Co., California. *Indicates a significant difference (t-test, $P < 0.05$).

Tree	Number	Length* (mm)	Width* (mm)	Area* (cm ²)	Dry weight (g)	DW/area* (g cm ⁻²)
Normal	32	75.5 (19.7)	35.2 (11.2)	21.9 (11.2)	0.23 (0.13)	0.010 (0.001)
Mutant	20	92.0 (20.4)	15.6 (4.3)	13.6 (5.4)	0.18 (0.07)	0.013 (0.001)

TABLE 3. COMPARISON OF STOMATAL DENSITIES (PER MM²) FOR LEAVES FROM A NORMAL AND MUTANT TANOAK TREE. Challenge Experimental Forest, Yuba Co., California.

Leaf side	Number	Normal	Mutant	t-test	P
Upper	9	4.6 ± 2.0	2.8 ± 2.0	-0.65	0.52
Lower	9	358.6 ± 28.0	105.5 ± 40.0	-4.64	0.0003

water loss, and possibly maintain a more favorable leaf-water status for continued carbon assimilation. This would become particularly important during the hot, dry summers typical

of the Sierra Nevada in the Challenge Experimental Forest area, but less so in the deep-shade environment of the sample trees. Leaf-level carbon assimilation is also dependent on nutrient status, and the lack of statistical differences between the two tanoak types for nitrogen, phosphorous, and potassium indicates no advantage for either type. The effects of significantly lower amounts of some of the minor elements and heavier metals in the mutant tanoak are unknown.

Physiological Functions

We found that maximum net photosynthesis (maximum assimilation rate, A_{max}) differed substantially between the mutant and normal tanoak trees (Fig. 6). A_{max} was $2.51 \mu\text{mol m}^{-2} \text{s}^{-1}$ for leaves from the mutant tree and $1.19 \mu\text{mol m}^{-2} \text{s}^{-1}$ for those of the normal tanoak. The light compensation point and dark respiration were $12.84 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $-0.34 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively, for leaves from the mutant tree and $15.63 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $-0.22 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively, for leaves of the normal tanoak. Quantum yield (the efficiency with which incoming



FIG. 4. Microphotograph of trichomes on mutant tanoak leaves (upper) and normal leaves (lower). It is interesting that the mutant condition greatly affected the size and shape of the leaves, but not the size and shape of the trichomes.

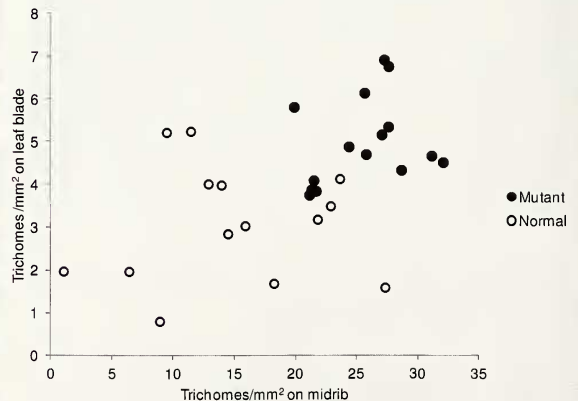


FIG. 5. Relationship of trichomes on blade and midrib of mutant and normal tanoak leaves.

TABLE 4. MEAN NUTRIENT CONTENT (AND STANDARD DEVIATION) OF LEAVES FROM A MUTANT AND NORMAL TANOAK TREE. Challenge Experimental Forest, Yuba Co., California.

Nutrient	Mutant	Normal
N (%)	1.10 (0.16)	1.14 (0.04)
P (%)	0.05 (0.001)	0.05 (0.002)
K (%)	0.48 (0.02)	0.43 (0.03)
Ca (%)	0.78 (0.03)	1.00 (0.11)
Mg (%)	0.14 (0.01)	0.15 (0.01)
S (%)	0.09 (0.01)	0.10 (0.01)
Al (ppm)	98.0 (18.7)	151.0 (60.7)
B (ppm)	25.2 (0.8)	45.8 (5.9)
Cu (ppm)	5.3 (0.56)	19.3 (14.5)
Fe (ppm)	119.0 (21.0)	164.0 (52.0)
Mn (ppm)	1124.0 (33.0)	2685.0 (157.0)
Na (ppm)	13.9 (1.2)	19.9 (6.6)
Zn (ppm)	14.4 (0.6)	26.6 (6.4)

light is converted to carbohydrates within a leaf) was higher in mutant leaves (0.028 mol CO_2) (mol incident photon) $^{-1}$ than in the leaves of the normal tanoak (0.015 mol CO_2) (mol incident photon) $^{-1}$.

Internal water potential was much more negative in the mutant tree than in the normal tree from 11:00–13:00 PST (Fig. 7). Both tanoak trees recovered at 14:00 PST. Considering a higher A_{max} in the mutant than in the normal tanoak, this suggests that the mutant tanoak is more tolerant of internal water stress than the normal tanoak.

Higher A_{max} and quantum yield, and a lower light compensation point in the mutant tanoak than in the normal type, indicates that the mutant tanoak is more efficient not only in capturing light from sun flecks under the tree canopy, but also at high PAR (photosynthetically active radiation) for carbon gain (Fig. 6). Furthermore, the mutant showed more negative leaf-water

potential than in the normal type (Fig. 7), which suggests a higher tolerance for water stress in the mutant.

Genetic Properties

Metabolomics. More than 220 individual metabolites were profiled using mass spectrometry metabolomic analyses. Of these, 41 were identified and the rest were unidentified peaks. Each peak is the result of the mass spectrometer detecting a different potentially unidentified metabolite. Because of the large number of metabolites, a multivariate approach, and particularly a principal component analysis (PCA) was used. Each PC explains a certain percent of the variation in the metabolite data. PC1 explained 26% of the variation in the data, while PC2 explained 18% (Fig. 8). The analysis showed substantial differences between the single mutant and the three normal trees, particularly along the second principal component (PC2). Metabolites that loaded positively onto PC2 included myo-inositol, xylonic acid, gluconic acid, phosphoric acid,

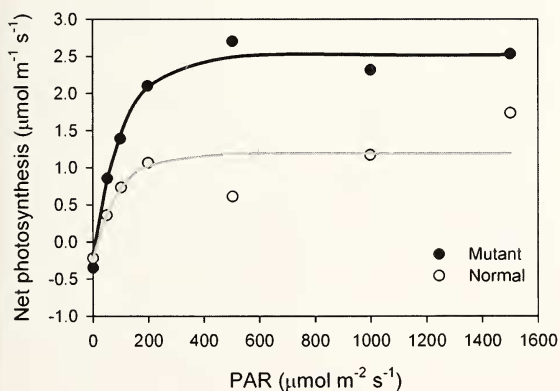


FIG. 6. Net photosynthesis ($n = 2$) at different photosynthetically active radiation levels for two leaves on mutant and normal tanoak trees on the Challenge Experimental Forest, Yuba Co., California. Lines are modeled with Hanson et al. (1987)'s equation. The relationship between measured and modeled values was $r^2 = 0.98$, $P < 0.001$ for the mutant leaves and 0.73 , $P < 0.05$ for the normal leaves.

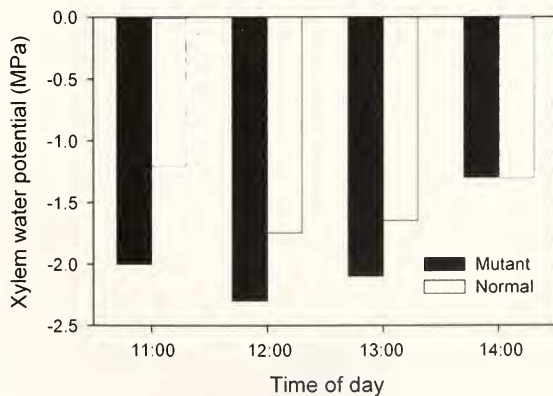


FIG. 7. Water potential of leaves on small twigs from mutant and normal tanoak trees at four sampling times, Challenge Experimental Forest, Yuba Co., California.

lactobionic acid, ribitol, serine, and isoleucine, along with a number that were unidentified.

Most classical genetic studies depend on the ability to create crosses, and in this instance crosses would be the most efficient means of understanding the genetics underlying the mutation. Since the mutants do not produce acorns, this technique is not possible; however, other tools are available that do not rely on crosses. The relatively new technique of metabolomics identified the metabolites in the tissue of each tanoak type. Analysis of the leaves of the two tanoak types showed very different metabolic profiles, even with similar tissue being collected in close proximity at the same time. Because metabolites are the product of proteins and enzymes that in turn are produced by RNA (and thus DNA sequences), these different metabolic profiles suggest that genetic differences exist between the two tanoak types. Because of the fluid nature of metabolic pathways, it is difficult to predict exactly which genes contain the genetic differences. The next step would be more genetic research, and particularly an analysis of the transcriptome (all of the mRNA or expressed genes in the tissue). This would help identify the genes that were associated with the changes in metabolites observed in this study.

Microsatellite variation. Based on the foliar sample from the 30 tanoak trees in the surrounding area, their DNA tells us that the mutant tanoak tree is similar to other local tanoaks and thus truly is a mutant in its genome, and not a hybrid or some other aberration. Both the mutant tree and normal forms were haplotype E, typical for tanoak trees elsewhere in the Sierra Nevada (Nettel et al. 2009). Nuclear microsatellite alleles did not differ between the mutant and the normal trees in the surrounding forest.

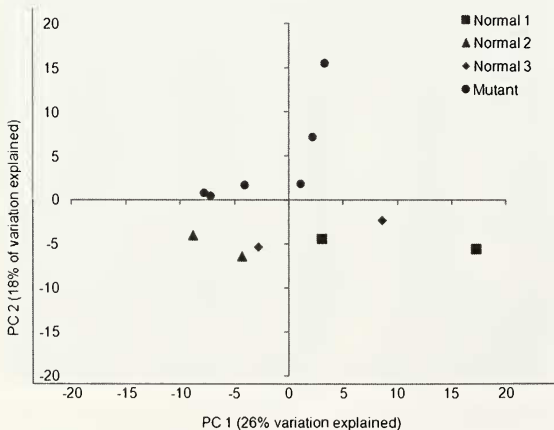


FIG. 8. Principal component analysis of metabolomic data based on two leaves from three normal tanoak trees and six leaves from the mutant tree.

Results of the nuclear and chloroplast markers indicate that the mutant is not a hybrid and that it originated in the local population. If there had been some hybridization or long-distance gene flow, we would expect to see a different genotype at the chloroplast loci. Instead, the mutant was observed to be the local haplotype. Also, because only a small number of microsatellite markers were used for our analysis of putatively neutral genetic variation, it is unlikely that our markers would be at or near the genetic mutation. In the future, a more complete genomic analysis may allow the specific mutation to be identified.

This result lends support to the theory that a mutation occurred in one or more genes that produced the unique phenotype. The most likely scenario is that the mutation occurs at a low frequency across the population. The mutant phenotype appears only in homozygous individuals, and heterozygous trees are fully fertile. Homozygous mutant offspring are continuously produced, but because of their metabolic differences, are unable to germinate and/or to produce seedlings except on rare occasions. And even when seedlings are produced, they tend to be weak and short-lived.

In 1994, mutant tanoak seedlings were found at two widely separated locations on the Klamath National Forest. Nine plants were found at the first location and 16 at the other. All were seedlings 2–45 cm tall. In 2009, none could be found. This supports the idea of a recessive, near-lethal gene or genes that are being preserved in the population. It might even suggest heterosis—the higher fitness of heterozygous individuals that preserve the allele at a higher frequency than might be expected.

Relative to SOD, post-inoculation lesion growth was similar in the mutant and normal tanoak leaves; hence there was no indication of a difference in susceptibility to SOD. However, research on SOD is continuing and is both intensive and extensive. A continuing effort seeks to find tanoak trees with natural resistance, but none have been found to date (Hayden et al. 2011).

A possible avenue of research on SOD involves the leaf trichomes. The inoculation technique that was used involved introduction of the inoculum through a cut in the leaf petiole. Suppose the inoculum was applied over the leaf and hence above the trichomes. This begs the question if so doing prevents spores from actually reaching the leaf surface, which would be beneficial, or would the trichomes hold water closer to the leaf surface, which would be a negative because it would aid penetration through the leaf tissue.

Enigmas

Grant (1971) noted that an evolutionary species has “its evolutionary tendencies, being

susceptible to change in evolutionary role during the course of its history.” Perhaps, “evolutionary tendencies” suggests that the species evolves into many forms and functions as it copes with an ever-changing environment during the millions of years of its existence on the planet. It might even be possible that the mutant will someday become well-adapted and reproduce. Enigmas could well be part of the evolutionary process.

Adaptation to drought is a major characteristic of the broad sclerophylls, and tanoak has many morphological and physiological structures and functions that promote this adaptation (McDonald 1982). However, its current natural range is characterized by a cool, moist climate with high levels of rainfall, fog, or relative humidity, not an environment that would seem to favor adaptation to drought.

Other enigmas concern the shrub form of tanoak as well as an instance of curious demise. The recognized shrub form, *N. densiflorus* var. *echinoides* (R. Br.) Manos, Cannon & S. H. Oh, in the mountains of northern California and southern Oregon is erect and has small leaves. It occurs “on high mountains” (Sudworth 1967), “on dry slopes from 610 to 2440 m” (Munz and Keck 1959), and between 600–2000 m (Tucker 2012). It is also found on much better and moister sites in the northern Sierra Nevada at the 1370–1525 m elevation, where it produces robust clumps of stems after cutting or burning (McDonald and Litton 1993). These grow upright for a few years, lean over, and then straggle downslope for 5 m or more. Here, the leaves are much larger than those of counterparts on poor sites, and are as large or larger than those of the tree form at the 760–1065 m elevation.

The curious demise took place on the Challenge Experimental Forest in an area having many normal and unusual clumps of tanoak sprouts from parent-tree root crowns. All clumps showed two or three generations of sprouting. Here, McDonald et al. (1988) sampled 19 clumps characterized by chlorotic leaf color, an abnormally large number of sprouts, a vastly different height-width relationship than healthy sprout clumps nearby, and a peculiar flat top with no tendency toward dominance by any sprout. After much testing, no pathogens or viruses that could account for the abnormal development were found in field or laboratory, and the reason for the decline and eventual death of the clumps was unknown.

On a much smaller scale, some morphological and physiological adaptations of tanoaks are enigmatic. For example, tanoak stomata are buried in crypts below the leaf surface and as noted earlier, have thousands of trichomes above. Both serve to reduce transpiration, and one would expect seedlings to be drought tolerant. However, research on six-year-old tanoak seed-

lings planted into a common garden on the Challenge Experimental Forest has shown a curious pattern of internal moisture stress during the summer. The seedlings were planted into an open field, under full sunlight. At the first hint of light in the morning, most stomata open most of the way (McDonald and Tappeiner 2002). This rapidly increases transpiration to the point that the loss in internal moisture exceeds the seedling’s daylight recharge capability—leading to eventual death. Efficient recharge ability at night only prolonged the process in these exposed seedlings. It appears that the inability to control the timing and degree of stomata opening trumps the morphological adaptations.

The timing, size, and condition of tanoak seed crops represent additional enigmas. It is likely that very few seed crops produce mutants, and we have no idea when the next will occur. Several complex transactions such as the interplay of high and low temperatures in the spring, selfing, ovule abortion, and others, could be a part of the process of mutant formation.

Seed crops of tanoak were quantified each year on the Challenge Experimental Forest from 1958–1981 (McDonald 1992). Seed was produced in 13 of the 24 yrs of record and amounted to three crops rated as very light, six as light, two as moderate, and two as heavy. A very light seed crop was recorded in 1958, and it is possible that the first mutant seedlings found in 1962 could have come from that crop.

We do know that mutants existed for at least four years beneath one mother tree and all were gone five years later. Anecdotal evidence suggests that not more than 50 mutant tanoak seedlings at least one year old were found at Challenge from 1962–2009.

Status

Because the mutant was so rare and its existence so precarious, cuttings from a short shrub at Challenge were sent to various arboreta and public gardens in California and Washington as insurance for its continued existence (W. Sundahl, USDA-Forest Service, Pacific Southwest Research Station, personal communication). Of those sent, many died or all records of them were lost. The most successful propagation was conducted by Arthur and Maren Kruckeberg at the MsF Rare Plant Nursery (now Kruckeberg Botanic Garden) near Shoreline, Washington. These cuttings were rooted with difficulty, and grown into small bushes or trees. Cuttings were then taken from them, rooted, and sold far and wide over the years (A. Kruckeberg, Univ. of Washington, personal communication). Two trees from the original propagation are now about 15 m tall and 12 m wide, and reside in the Botanic Garden. Healthy mutant tanoak trees

have been reported from several locations in western Washington, Oregon, and California, as well as Great Britain and the Netherlands. All successful propagations reside in well-tended, park-like settings having rich, fertile soil with plentiful water and shade. A few other nurseries also root cuttings and sell them, but none had specimens for sale in their 2009 catalogs. Of importance is that all the mutant tanoaks that are known originally came from the Challenge Experimental Forest.

We have shown that the mutant tanoak seems to be as well adapted to the narrow environment of deep shade and fertile soil as its normal counterpart nearby. However, evidence suggests that mutant tanoak seedlings cannot begin life and grow well in a less benign environment. Even in tended gardens, the cuttings must have shade, deep soil, and possibly fertilizer when young.

The odds of this mutant ever becoming a viable species are low. If the mutant could get past the initial sterility and inviability bottlenecks, it would have difficulty becoming established in a thriving population of non-mutant individuals (Grant 1971).

Daubenmire (1959) noted "Of the thousands of genes that govern the behavior of an organism, only one needs to change beyond a certain extent in order to disturb the synchrony of the various functions and thereby prove fatal. The vast majority of genetic variations are probably unsuccessful because the physiologic balance has been upset by the new combination of genes. Thus there is an internal requirement for harmony in addition to the demand for harmony between the new gene complex and the environment."

As noted earlier, tanoak is regarded as an evolutionary species, meaning as Grant (1971) suggests that it occupies an ecological niche of its own in nature for which it is especially adapted.

TAXONOMIC TREATMENT

Notholithocarpus densiflorus (Hook. & Arn.) Manos, Cannon & S. H. Oh f. **attenuato-dentatus** (J. M. Tucker, Sundahl, & D. O. Hall) McDonald, Zhang, Senock & Wright comb. nov. Fig. 1. Basionym: *Lithocarpus densiflorus* (Hook. & Arn.) Rehder f. *attenuato-dentatus* J. M. Tucker, Sundahl, & D. O. Hall, *Madroño* 20:224–225. 1969.

CONCLUSIONS

Both normal tanoak and its mutant present many enigmas. We have endeavored to study and express some of the physical, morphological, physiological, and genetic attributes of both types of tanoak. We have noted several enigmas in the

normal tanoak, which potentially suggest that it has changed both morphologically and physiologically during its millions of years of existence on the planet. These changes are likely a response to an ever-changing climate. Our efforts have been both extensive and intensive, and we have gained in knowledge. To paraphrase a noted novelist: "Knowledge is like a river; the deeper it is, the less noise it makes." Perhaps, a bit more noise in the form of more research, especially in genetics, is needed. In that sense, our work here is a base from which that work can proceed.

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